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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/120,044	07/21/1998	CONCEICAO MINETTI	3842-4036US2	1759

7590 05/06/2003

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EXAMINER
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ART UNIT	PAPER NUMBER
1645	40

DATE MAILED: 05/06/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
09/120,044	Minetti et al.
Examiner	**Art Unit**
S. Devi, Ph.D.	1645

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *three* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on *Feb 14, 2003*.

2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *35-37, 42-51, 53, and 60-80* is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5) ☒ Claim(s) *43-51* is/are allowed.

6) ☒ Claim(s) *35-37, 42, 53, and 60-80* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). *36.*
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

RESPONSE TO APPLICANTS' AMENDMENT

Applicants' Amendment

1) Acknowledgment is made of Applicants' amendment filed 02/14/03 (paper no. 39) in response to the non-final Office Action mailed 08/14/02 (paper no. 37).

Status of Claims

2) Claims 35, 53, 62 and 64-79 have been amended via the amendment filed 02/14/03.

New claim 80 has been added via the amendment filed 02/14/03.

Claims 35-37, 42-51, 53 and 60-80 are under examination.

Prior Citation of Title 35 Sections

3) The text of those sections of Title 35 U.S. Code not included in this action can be found in a prior Office Action.

Prior Citation of References

4) The references cited or used as prior art in support of one or more rejections in the instant Office Action and not included on an attached form PTO-892 or form PTO-1449 have been previously cited and made of record.

Objection(s) Withdrawn

5) The objection to claim 73 made in paragraph 23 of the Office Action mailed 08/14/02 (paper no. 37) is withdrawn in light of Applicants' amendment to the claim.

6) The objection to the specification made in paragraph 8(a) of the Office Action mailed 08/14/02 (paper no. 37) is withdrawn in light of Applicants' amendment to the specification.

7) The objection to the specification made in paragraph 8(b) of the Office Action mailed 08/14/02 (paper no. 37) is withdrawn in light of Applicants' amendment to the specification.

Objection(s) Maintained

8) The objection to the drawings made in paragraph 7 of the Office Action mailed 08/14/02 (paper no. 37) is maintained for reasons set forth therein.

Rejection(s) Withdrawn

9) The rejection of claims 35-37, 42, 53 and 60-79 made in paragraph 22 of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, first paragraph, as containing new subject matter, is withdrawn in light of Applicants' amendment to the claims and/or the base claim(s).

Searching for functionally similar sequences

To search for functionally similar sequences, use the "family" search options: Family Exact Sequence Search (/SQEFP), and Family Subsequence Search (/SQSFP). In family searches, each common amino acid in the query has to match either the exact amino acid or a functionally similar "equivalent," as shown in the following table.

Property	Functionally Similar Amino Acids
Neutral-Weakly Hydrophobic	Ala,Gly,Pro,Ser,Thr (A, G, P, S, T)
Hydrophilic-Acid Amine	Asn, Asp, Gln, Glu (N, D, Q, E)
Hydrophilic-Basic	Arg,His,Lys (R, H, K)
Hydrophobic	Ile,Met,Leu,Val (I, M, L, V)
Hydrophobic-Aromatic	Phe,Trp,Tyr (F, W, Y)
Crosslinking	Cys (C)

10) The rejection of claims 69-79 made in paragraph 23 of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, first paragraph, as containing new subject matter, is withdrawn in light of Applicants' amendment to the base claim.

11) The rejection of claim 35 made in paragraph 25(a) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

12) The rejection of claim 35 made in paragraph 25(b) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

13) The rejection of claim 53 made in paragraph 25(c) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

14) The rejection of claim 62 made in paragraph 25(d) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

15) The rejection of claim 64 made in paragraph 25(e) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

16) The rejection of claim 65 made in paragraph 25(f) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

17) The rejection of claim 65 made in paragraph 25(g) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

18) The rejection of claim 66 made in paragraph 25(h) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

19) The rejection of claim 66 made in paragraph 25(i) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of

Applicants' amendment to the claim.

20) The rejection of claims 67 and 68 made in paragraph 25(j) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claims.

21) The rejection of claims 65-69, 71-73, 75, 77 and 78 made in paragraph 25(k) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claims.

22) The rejection of claims 70, 72, 74, 76 and 79 made in paragraph 25(l) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claims.

23) The rejection of claims 36, 37, 42, 53 and 60-79 made in paragraph 25(m) of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claims.

24) The rejection of claim 53 made in paragraph 24 of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C § 112, first paragraph, as being non-enabled, with regard to the scope, is withdrawn in light of Applicants' amendment to the claim.

Rejection(s) Maintained

25) The rejection of claims 35-37, 42 and 60-79 made in paragraph 24 of the Office Action mailed 08/14/02 (paper no. 37) under 35 U.S.C § 112, first paragraph, as being non-enabled, with regard to the scope, is maintained for part of the reasons set forth therein and herebelow. Claim 80 is now included in this rejection.

It is noted that Applicants have amended claim 35 which addresses several issues raised in the rejection. On page 19 of the amendment filed 02/14/03, Applicants state that claim 35 "does not include a modified pneumolysin having substituted amino acids at position 243 alone". Applicants assert that the modified pneumolysin polypeptide having an amino acid substitution at position 243 "must also have" at least one other substitution at positions 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 255 and 257. From these statements, it appears that Applicants' intention is to claim a modified pneumolysin polypeptide that has an amino acid substitution at position 61, 148 or 195 of the amino acid sequence of SEQ ID NO: 3, alone or in combination with

at least one amino acid substitution in the amino acid sequence of SEQ ID NO: 3 at a position selected from the group consisting of position 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255 and 257, wherein the modified polypeptide has the recited three functions. If so, then Applicants should consider amending claim 35 as suggested at the end of this Office Action. Claim 35, as amended currently, is internally inconsistent and does not convey this meaning. The same is true with claim 80. The recitation "at least one" in line 2 of claim 35 indicates that at least two types of modified pneumolysin polypeptides are encompassed in the scope of the claim: (i) a modified pneumolysin carrying only one amino acid substitution at position 61, 148 or 195 of the amino acid sequence of SEQ ID NO: 3; and (ii) a modified pneumolysin carrying one or more amino acid substitutions at positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255 and 257, wherein said modified pneumolysin has the three recited functions. A reasonable interpretation of claim 35 is that when the only one amino acid substitution is not at position 61, 148 or 195, the substitution can be at position 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 148, 189, 239, 243, 255 and 257. All these modified pneumolysins are required to be soluble, required to elicit antibodies cross-reactive with wild-type pneumolysin and required to have attenuated hemolytic activity. However, it is evident from Applicants' own description in the instant specification that these modified pneumolysins constitute significant numbers of inoperative embodiments, because the specification expressly describes that an amino acid substitution at position 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 255 or 257 of SEQ ID NO: 3 alone does **not** reduce (i.e., attenuate) the hemolytic activity of the pneumolysin, because these sites are **not** associated with decreases in hemolytic activity. See the sentences pages 25 ans 26 and the one on page 26 of the specification which are reproduced herebelow:

> Non-limiting examples of amino acid residues which may be substituted but **which alone do not reduce hemolytic activity** include those at positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 127, 128, 172, 189, 239, 255 and 257. Examples of substitutions at these positions include, but are not limited to those shown in Table 3..... [Emphasis added]

The last paragraph on page 8 of the specification also describes that a single specific amino acid substitution at one of these positions did not attenuate the hemolytic activity. See also lines 1-9 on page 26 of the specification. Therefore, at least one of the recited functions in these single mutants which are currently encompassed in the scope of the claim is contrary to what is being recited.

Contrary to the situation in *In re Angstadt, 537 F.2d 498, 502-03, 190 USPQ 214, 218 (CCPA 1976)*, wherein a disclosure of a large number of operable embodiments and the identification of a single inoperative embodiment was found not to render a claim broader than the enabled scope, in the instant case, claims encompass a significant number of demonstrated inoperative embodiments, i.e., modified pneumolysins that are demonstrated within the instant specification to possess functions contrary to what is recited. In the instant case, the disclosure is not commensurate with the scope of the protection sought by the base claim.

With regard to the teachings of Lazar *et al.* which was cited by the Office to establish the art-recognized structure-functional unpredictability even when an amino acid in a polypeptide is substituted with another conservative amino acid, Applicants allege that the Office is attempting to mix and match different combination of characteristics to refute the possibility of substituting amino acids which result in similar functions. Applicants appear to dismiss Lazar *et al.* and Hansen *et al.* by stating that these references refer to one characteristic and not the entire invention as claimed. However, at least with regard to Lazar's substitution of leucine with isoleucine in the TGF alpha polypeptide, Applicants acknowledge that leucine to isoleucine is a conservative substitution, and assert that 'modified pneumolysin polypeptides having conservative amino acid substitutions that are soluble, capable of eliciting antibodies which are cross-reactive with wild-type pneumolysin and have reduced hemolytic activity' are provided (see page 27 of Applicants' amendment filed 02/14/03). Applicants allege that Hansen's substitution of aspartic acid with asparagine is not equivalent. However, contrary to Applicants' assertion, the art recognizes aspartic acid and asparagine to be functionally similar amino acids having hydrophilic acid amine property (see the attached page 12 from 'Protein Sequences on STN). Lazar *et al.* (*Mol. Cellular Biol.* 8: 1247-1252, 1988, already of record) demonstrated that a substitution of Leu with a conservative amino acid, such as, Ile, in the transforming growth factor (TGF) alpha led to a mutant protein with dramatically altered biological activities. Lazar *et al.* expressly stated that they "did **not** expect that a mutation of Leu to Ile (which have similar sizes and polarities) would cause such a strong effect". See paragraph bridging left and right columns on page 1251; and third full paragraph on page 1251 of Lazar *et al.* Similarly, it is well known in the art that a single amino acid substitution even with a functionally equivalent amino acid can drastically alter a peptide's biologic or antigenic function as well. Hansen *et al.* (WO

98/06851 - already of record), a post-filing reference, disclosed that when hydrophilic amino acid, aspartic acid (D), is substituted for another hydrophilic amino acid, asparagine (N), at position 3 of the peptide, LDNKYAGKGY, the reactivity of the peptide with the bactericidal 10F3 monoclonal was abolished. See lines 8-14 of WO 98/06851 of Hansen *et al.* It is noted that the instant specification at lines 8-11 of page 34 describes conservative amino acid substitutions as representing substitutions of "similar" amino acids and that they are not limited to polarity, hydrophobicity, size and side chain structure. Thus, from Applicants' own description it is evident that the charge of an amino acid at neutral pH is not the only criteria that determines whether or not an amino acid is conservative or non-conservative.

With regard to the scope of claims 65-67, Tables 8 and 9 provide enabling disclosure for single site-modified pneumolysin polypeptides having $Phe^{195}Val$ substitution, $Met^{148}Lys$ substitution, $Phe^{195}Ile$ substitution and $Ser^{61}Pro$ substitution which possess the recited functions. However, the claimed polypeptide encompasses pneumolysin single mutants having hydroxyproline at position 61; arginine or histidine at position 148; leucine, glycine or alanine at position 195; arginine, valine, glutamic acid or serine at position 243; serine, asparagine, glutamine, tyrosine and cysteine at position 33 and 46; threonine, asparagine, glutamine, tyrosine and cysteine at position 83; lysine and histidine at position 239; and leucine, alanine, isoleucine and valine at position 257. In cases involving unpredictable factors, it cannot be predicted from the disclosure of a single specific amino acid substituted species at position 61, 148 or 195, what other substituted species will work. Similarly, claim 77 is unfoundedly broad and is enabled only for the specific modified pneumolysin polypeptide species having a threonine substitution at position 33 and 46; serine substitution at position 83; arginine substitution at position 239; and glycine substitution at position 257. What are claimed in claims 65-67 and 77 are specific species which are required to have each of the three functions recited in the base claim. However, Applicants did not have possession of these modified pneumolysin species, which species possessed the three required or recited functions. Given the prophylactic or vaccine application of the claimed modified pneumolysin product and the breadth of the instant claims, Lazar's and Hansen's demonstration of unpredictability with regard to the structure-function association in a polypeptide is critically important. With this demonstration, it is reasonable for one of skill in the art to conclude that the non-enabled modified pneumolysin species

specifically claimed in claims 65-67, including those wherein the hydrophobic Met is substituted with a non-conservative basic-hydrophilic Arg or His; and those wherein the hydrophobic- aromatic Phe is substituted with a non-conservative hydrophobic Leu or weakly hydrophobic-neutral Gly or Ala, would not result in a modified pneumolysin that retains all the three distinct functions recited: solubility; attenuated hemolytic activity; and ability to elicit antibodies that are cross-reactive with the wild-type pneumolysin. Given Lazar's and Hansen's showing that retention of a single function of a polypeptide after a single conservative amino acid substitution is highly unpredictable, it is reasonable for one skilled in this art to conclude that retention of more than one function along with the acquisition of the desired attenuation in hemolytic activity following one or more conservative or non-conservative amino acid substitutions in pneumolysin polypeptide, would not have been predictable, absent a concrete showing. Regardless of the complexity or simplicity of the method of isolation and method of testing, conception cannot be achieved until reduction to practice has occurred. Applicants are reminded that conception of one species within a genus may not constitute conception of another species in the genus. *Oka v. Youssefyeh, 849 F.2d 581, 7 USPQ2d 1169 (Fed. Cir. 1988)*. Adequate enablement requires more than a mere statement that its is a part of the invention and a reference to a potential method of isolating or testing it. Whether or not Applicants are required to disclose every species encompassed in the scope of a broad or generic claim even in an unpredictable art is not the issue with regard to claims 65-67 and 77. What is at issue here is, in light of the unpredictability disclosed within the instant disclosure and within the art, whether or not Applicants have enabled and/or were in possession of every modified pneumolysin species that is being 'specifically' claimed, for example, in claims 65-67 and 77, wherein the specific species are required to possess the three specific functions recited in the base claim. The courts have held that it is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement. See *Genentech Inc., v. Novo Nordisk A/S Ltd., 42 USPQd 1001*. Moreover, the specification must have been enabling at the time the invention was made and developments after the time of filing are of no consequence to what one skilled in the art would have believed at the time of filing (*In re Wright, 27 USPQ2d 1510, Federal Ceircuit, 1993*).

With regard to claim 75, if the modified pneumolysin polypeptide of claim 35 "must" have an

amino acid substitution at position 61, 148 or 195 as Applicants insist, then claim 75 cannot depend from claim 35, because the modified pneumolysin claimed in claim 75 does not contain the primary amino acid substitution at position 61, 148 or 195. Moreover, the pneumolysin species of claim 75 has not been shown to possess all the three functions recited in claim 35. Applicants should consider presenting claim 75 as an independent claim as suggested at the end of this Office Action.

Applicants' arguments have been carefully considered but are only partly persuasive.

New Rejection(s)

Applicants are asked to note the following new rejection(s) made in this Office Action. The new rejections are necessitated by Applicants' amendments to the claims and/or the base claim and/or the submission of new claim(s).

Rejection(s) under 35 U.S.C. § 112, Second Paragraph

25) Claims 35-37, 42 and 60-80 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(a) Claim 35 is vague, indefinite, confusing and internally inconsistent in the recitation: 'comprises substituting at least one amino acid ..... selected from the group consisting of .. 17, 18, 33, 41, 45, 46, .. 66, 83, 101, 102, 128, ... 189, ... 239, 255 and 257..... wherein ... possesses only one substitution ..... positions 61, 148 and 195'. The term 'at least one' encompasses more than one. The modified pneumolysin in the second half of the claim is limited to having only one substitution at position 61, 148 or 195. Yet, the first half of the claim recites/encompasses one or more substitutions at positions other than position 61, 148 or 195. Because of this internal inconsistency, the metes and bounds of the claim are indeterminate. If Applicants' intention is to claim a modified pneumolysin polypeptide that has an amino acid substitution at position 61, 148 or 195 of the amino acid sequence of SEQ ID NO: 3, alone or in combination, with at least one amino acid substitution at a position of SEQ ID NO: 3 which position is selected from the group consisting of positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255 and 257, then Applicants should consider amending claim 35 as suggested at the end of this Office Action.

(b) Analogous criticism applies to the new claim 80. The metes and bounds of claim 80 are incomprehensible.

(c) Claim 35 is confusing, because it is unclear where does the antecedence come from for the recitation: "said modified pneumolysin", in lines 6 and 7 of the claim. It appears that at least two types of modified pneumolysin polypeptides are encompassed in the scope of the claim: a) a modified pneumolysin carrying only one amino acid substitution at position 61, 148 or 195 of the amino acid sequence of SEQ ID NO: 3; and b) a modified pneumolysin carrying one or more amino acid substitutions at positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255 and 257. From the above identified limitation "said modified pneumolysin" in lines 6 and 7 of the claim, it is unclear whether this 'said modified pneumolysin' is the one that has a single mutation at position 61, 148 or 195, or whether this 'said modified pneumolysin' is referring to the second type of the modified pneumolysin carrying one or more amino acid substitutions at positions 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255 and 257. It is particularly noted in this connection that the specification expressly describes that an amino acid substitution at position 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 255 or 257 of SEQ ID NO: 3 alone does **not** reduce (i.e., attenuate) the hemolytic activity of the pneumolysin

(d) Claim 65 is confusing in the recitation: "The polypeptide according to claim 35 .... said modified pneumolysin polypeptide". For clarity and for distinctly claiming the subject matter, it is suggested that Applicants replace the recitation with --The modified polypeptide according to claim 35, wherein said substitution at position 61 is a proline or hydroxyproline substitution--.

(e) Analogous criticism applies to claims 66-68.

(f) Claim 80 is vague, indefinite and confusing in the recitation "substituting at least one amino acid **sequence** having SEQ ID NO: 3", because it is unclear how and with what the whole sequence of SEQ ID NO: 3 is substituted [Emphasis added].

(g) Claim 80 is indefinite, confusing and/or improper in the recitation: "substitution is at a position selected from the group consisting of 17, 18, 33, 41, 45, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255 and 257 ..... wherein the amino acid substitution at position 127 .... wherein the amino acid substitution at position 172 ...". The latter recited position 127 or 172 is not recited earlier as one of the Markush species.

(h) Claim 80 is indefinite, confusing and is inconsistent with the disclosure, because the claim encompasses numerous modified pneumolysin polypeptides including those having one amino

acid substitution at a recited position which is other than position 61, 148 and 195 wherein the modified polypeptide is soluble, elicits antibodies cross-reactive with wild-type pneumolysin and has attenuated hemolytic activity. Such a modified pneumolysin polypeptide includes the one with an amino acid substitution at position 17, 18, 33, 41, 45, 46, 63, 66, 83, 101, 102, 128, 189, 239, 243, 255 or 257 of SEQ ID NO: 3 alone, which is explicitly described in the instant specification as not showing attenuated hemolytic activity. See the sentence bridging pages 25 and 26; and lines 1-9 on page 26 of the specification. Thus, the claim improperly includes several modified pneumolysin polypeptides not having one of the three recited and required functions. Furthermore, encompassed in the scope of the claim is a modified pneumolysin polypeptide having an amino acid substitution at position 243. However, the specification discloses that a mutation at position 243 of the wild-type pneumolysin either with arginine, valine, glutamic acid or serine, alone or in combination with additional mutations, resulted in exclusively insoluble species and that attempted refolding of these mutants yielded aggregate species (see pages 57 and 58).

(i) Claims 36-37, 42 and 60-79, which depend directly or indirectly, from claim 35, are also rejected under 35 U.S.C. § 112, second paragraph, as being indefinite, because of the vagueness or indefiniteness identified above in the base claim.

Rejection(s) under 35 U.S.C. 102

26) Claim 53 is rejected under 35 U.S.C. § 102(b) as being anticipated by Lock *et al.* (*Microb. Pathogen.* 21: 71-83, 1996 - Applicants' IDS), or Lee *et al.* (*Vaccine* 12: 875-878, 1994, already of record), or Paton *et al.* (*Infect. Immun.* 59: 2297-2304, 1991 - Applicants' IDS), or Alexander *et al.* (*Infect. Immun.* 62: 5683-5688, 1994, already of record).

It is noted that Applicants use the terms, pneumolysoid and modified pneumolysin, interchangeably in the instant specification (see page 6, lines 8 and 9, for example).

Lock *et al.* taught a modified pneumolysin, Ply8, having substantially reduced (i.e., attenuated) haemolytic activity compared to the wild type pneumolysin, Ply, as tested by a haemolytic assay. Ply 8, produced by the host cells, has a random single amino acid substitution (see abstract; and pages 75 and 80). The modified pneumolysin is in a phosphate buffer, i.e., a pharmaceutically acceptable carrier (see page 81). Lock *et al.* explicitly taught that antibodies "raised against Ply completely neutralize Ply8 haemolytic activity and **vice versa**" (see page 80)

(Emphasis added). That Ply8 was used to raise antibodies, which neutralize native Ply, clearly suggests that Lock's Ply8 is soluble. Lock *et al.* expressly taught that Ply toxoid (i.e., modified pneumolysin) is a promising candidate for inclusion in the next generation of pneumococcal vaccines (see page 78).

Lee *et al.* taught a Pd-B pneumolysoid, i.e., a modified and haemolytically-attenuated pneumolysin, carrying a random amino acid substitution, and its conjugation to serotype 19F pneumococcal polysaccharide (see paragraph bridging pages 875 and 876). The conjugate conferred protection against type 19F pneumococcal challenge in infant mice born to pregnant or lactating mothers immunized with the conjugate (see Materials and Methods; and Results and Discussion). The pneumolysoid elicited antibodies cross-reactive with the wild-type pneumolysin (see Table 1). The fact that Lee's pneumolysoid was used in a conjugation reaction to produce a conjugate vaccine is evidence that it was soluble.

Paton *et al.* taught modified pneumolysins or pneumolysoids or pneumolysin toxoids designated Pd-A and Pd-B, both carrying a random amino acid substitution (see abstract and page 2298, left column). The two pneumolysoids showed a reduced or attenuated hemolytic activity compared to that of native pneumolysin (see page 2299, right column, first paragraph under 'Results'). The pneumolysoids are produced by random site-directed mutagenesis or amino acid substitutions, which significantly reduce the hemolytic activity (see page 2298). The pneumolysoids are expressed by *E. coli* host cells and were assayed for hemolytic activity (see page 2298, right column, first paragraph). Both Pd-A and Pd-B were contained in a pharmaceutically acceptable carrier, such as PBS (see page 2299, left column). Paton *et al.* taught the conjugation of Pd-B to the pneumococcal serotype 19F polysaccharide and a vaccine comprising the same (see page 2299, left column). That Paton's Pd-B pneumolysoid (i.e., modified pneumolysin) having an attenuated haemolytic activity served as an effective immunogen, with and without conjugation to a polysaccharide, suggests that the single amino acid substitution did not disrupt the secondary structure critical for the conformational and functional integrity of the modified pneumolysin. The fact that Lee's pneumolysoid was used in a conjugation reaction to produce a conjugate vaccine (see paragraph bridging pages 2298 and 2299) is evidence that it was soluble.

Alexander *et al.* disclosed a pneumolysin toxoid (PdB) comprising an amino acid substitution

introduced by randomly mutating the nucleotide sequence of the pneumolysin gene. The PdB was dissolved in PBS, was less haemolytic and elicited antibodies that cross-reactive with the wild-type pneumolysin (see abstract; and pages 5684 and 5685). The fact that Alexander's pneumolysoid was used in a conjugation reaction to produce a conjugate vaccine also indicated that it was soluble.

Claim 53 is anticipated by Lock *et al.* or Lee *et al.* or Paton *et al.* or Alexander *et al.*

Remarks

27) Independent claims 43-51 are allowed. Claims 35-37, 42, 53 and 60-80 stand rejected.

It is noted that claims 71-74 depend from claim 35, yet improperly recite an amino acid position, for example 72 or 127, which is not a part of claim 35.

28) It is suggested that Applicants present the base claim 35 as shown below:

--35. An isolated modified pneumolysin polypeptide comprising an amino acid substitution at position 61, 148 or 195 of the amino acid sequence of SEQ ID NO: 3, alone or in combination with at least one amino acid substitution in said amino acid sequence of SEQ ID NO: 3 at a position selected from the group consisting of 17, 18, 41, 45, 63, 66, 101, 102, 127, 128, 172, 189, 243 and 255, wherein said modified pneumolysin is soluble, retains at least one epitope capable of binding an antibody specific to native pneumolysin, and is attenuated in hemolytic activity.--

It should be noted that amino acid positions 127 and 172 are added above in claim 35 as Markush positions, which would justify the dependency of claim 42 by providing proper antecedence for modified polypeptides, pNVJ20 and pNVJ45, each of which includes an amino acid substitution at position 127 or 172. The addition of positions 127 and 128 is also necessary to provide proper antecedence for the recitation "172" or "127" in claims 71-74.

Since the modified pneumolysin polypeptide claimed in claim 75 does not have a primary mutation in position 61, 148 or 195 as required by the proposed base claim 35, it is suggested that claim 75 be presented as an independent claim as follows:

--75. An isolated modified pneumolysin polypeptide comprising an amino acid substitution at positions 33, 46, 83, 239 and 257 of the amino acid sequence of SEQ ID NO: 3, wherein said modified pneumolysin is attenuated in hemolytic activity.--

29) Applicants' amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See M.P.E.P § 706.07(a). Applicants are reminded of the extension of time policy as set forth in 37 C.FR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

30) Papers related to this application may be submitted to Group 1600, AU 1645 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1. The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242, which is able to receive transmissions 24 hours a day and 7 days a week. The RightFax number for submission of before-final amendments is (703) 872-9306. The RightFax number for submission of after-final amendments is (703) 872-9307.

31) Any inquiry concerning this communication or earlier communication(s) from the Examiner should be directed to S. Devi, Ph.D., whose telephone number is (703) 308-9347. A message may be left on the Examiner's voice mail service. The Examiner can normally be reached on Monday to Friday from 7.15 a.m to 4.15 p.m. except one day each bi-week which would be disclosed on the Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

May, 2003

S. DEVI, PH.D.
PRIMARY EXAMINER